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NOTES ON THE GAMETOPHYTES AND EMBRYO OF PODOCARPUS.

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(WITH PLATES V–VII)

DURING a visit to Jamaica in the summer of 1900 I took advantage of the opportunity to make a collection of female cones of *Podocarpus coriacea* from trees cultivated in Castleton Garden. The climate of this part of the island, hot and wet, is apparently not congenial to this species, for it was afterward found that none of the ovules had been pollinated. This absence of pollen tubes, in connection with the fact that it was necessary to fix in alcohol, made the material of little value, and only a few of the figures are drawn from it.

Most of the results were obtained from collections made in June and July, 1901, from a male and a female tree grown in Darlington, South Carolina. The plants were small and scraggy, but both bore numerous cones, and, though they were separated by more than a hundred feet, over 95 per cent. of the ovules examined were furnished with pollen tubes. I cannot state positively that these trees are also *P. coriacea*, as there was no means of identifying them at the time, and I neglected to bring the vegetative parts to this country. However, as the fruits seem identical with those collected in Jamaica, it is probable that they are the same species.

It is unfortunate that the results here given are not more complete, and they are published with the hope of supplementing them from future collections. The Darlington material was fixed at the tree in corrosive-acetic (aqueous solution of corrosive sublimate 95 parts, glacial acetic acid 5 parts), the prothallium being exposed at the sides or removed with the nucellus from the integuments. Sections 5 or 10 μ in thickness were made in paraffin, and in the case of the pollen stained in Haidenhain's iron-alum-haematoxylin or Flemming's triple. For prothallium and archegonium the latter stain was most used; while with embryos a combination of Delafield's haematoxylin and saffranin gave the best results.

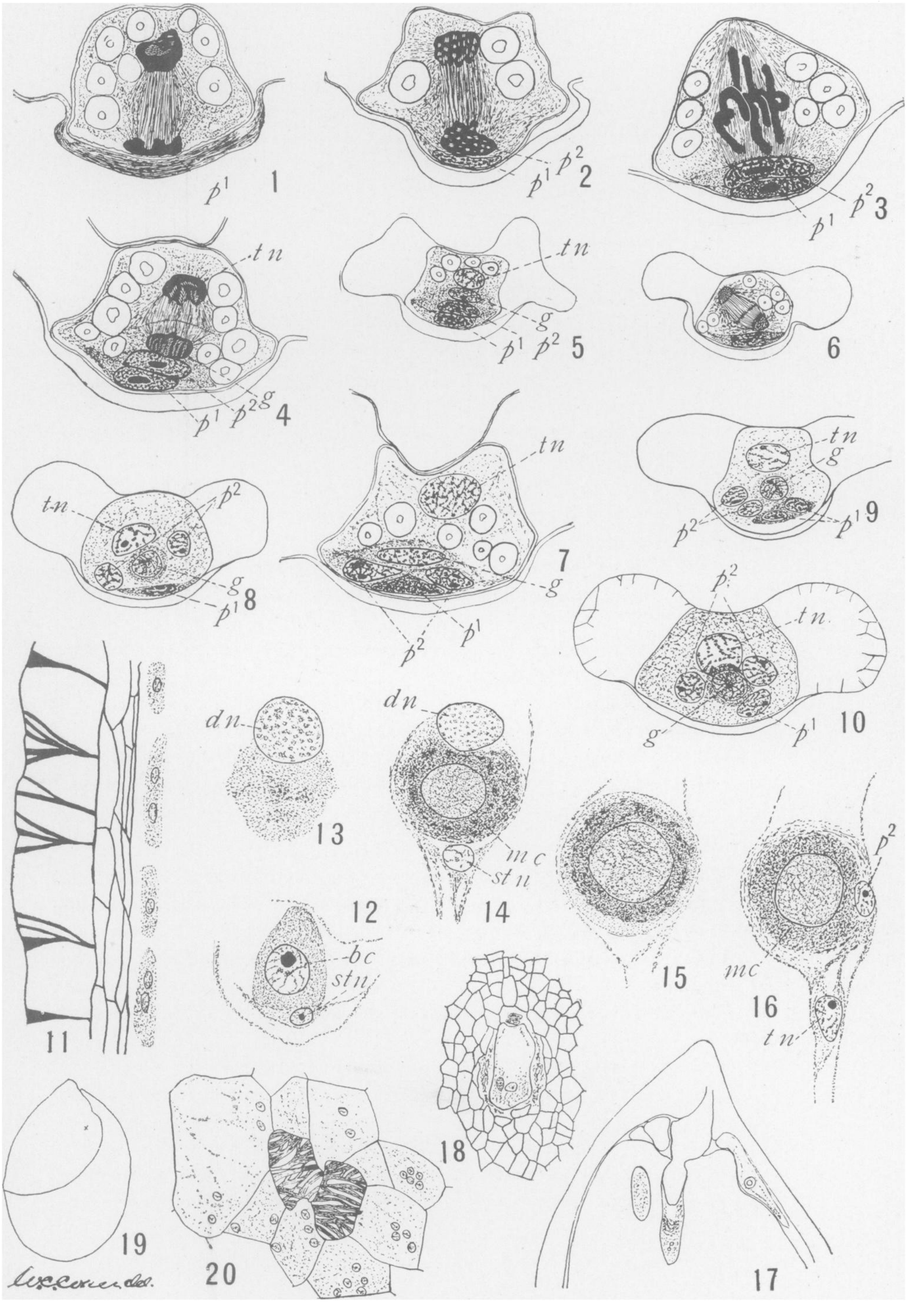
Most of the work has been done in the Bonn laboratory, and I wish to express here my appreciation of the unfailing kindness of Professor Strasburger.

THE POLLEN SAC AND POLLEN GRAIN.

Two pollen sacs are borne on the lower surface of each sporophyll, as in the Abieteae, but they differ from these in being less sunken in the tissue and in their wider separation. The wall of the mature sac (*fig. 11*) consists of an outer layer of large cells whose walls are strengthened internally with thickened bands, and of three or four layers of thin cells which finally almost completely collapse. Within these is the one-layered tapetum, which disorganizes during the time that the pollen grains are undergoing their divisions. In *fig. 11* some of the tapetal cells are shown to contain two nuclei, which is often the case. It will be noticed that the microsporangium wall as here described is very like that of the Abieteae, while it differs from the generally two-layered wall found in the Cupresseae and Taxodium.[1]

In the development of the pollen the youngest stage found was after the separation of the tetrads, and before the first division of the grain. The wings have already reached their full size. All stages, from the uninucleate to the completely mature

[1] All references to Taxodium, unless otherwise stated, are taken from my unpublished paper on the gametophyte and embryo of Taxodium, a short preliminary account of which appeared in the *Johns Hopkins University Circular* 19:—. 1900.



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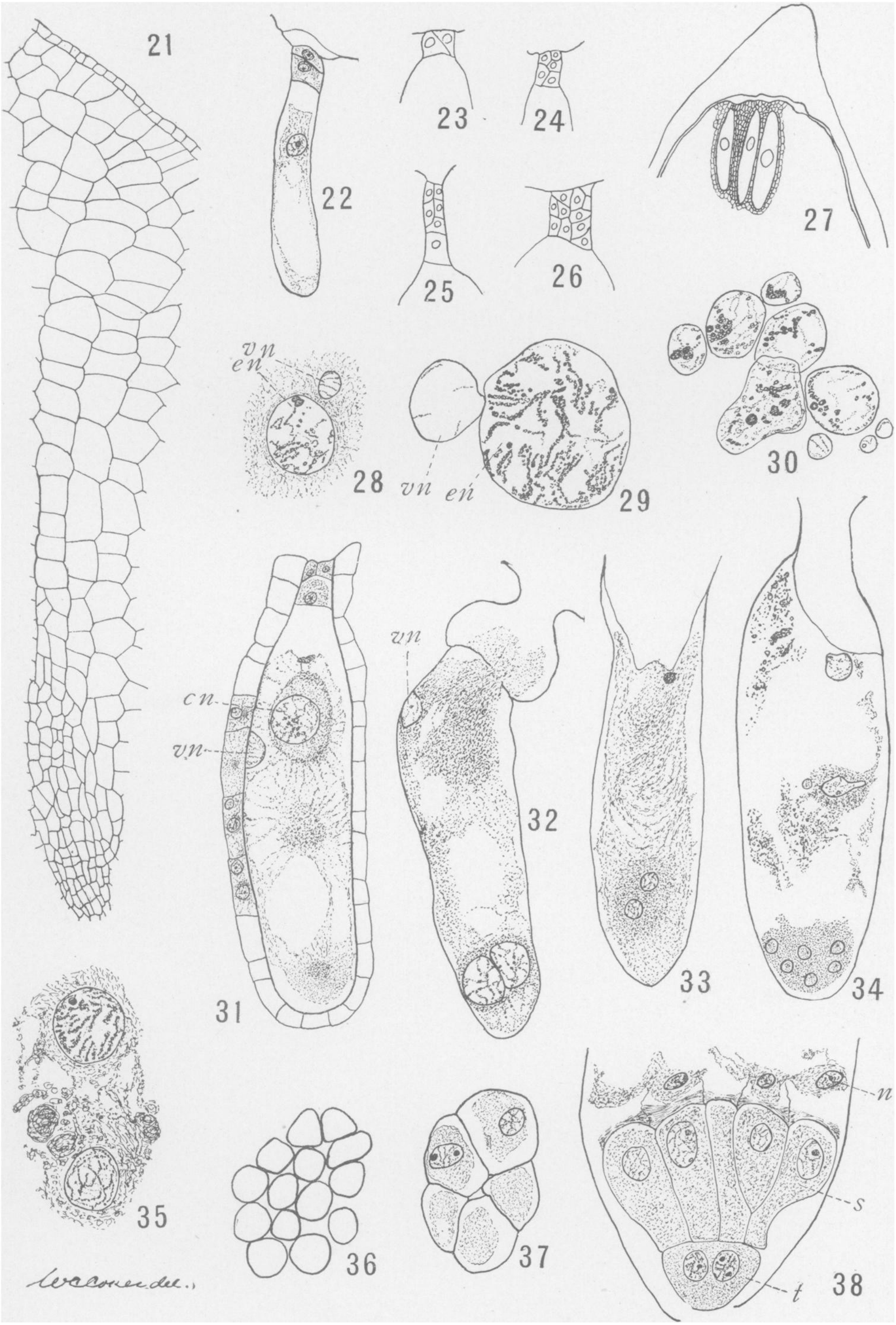

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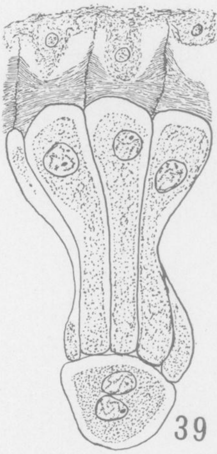

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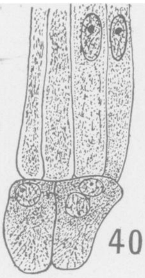

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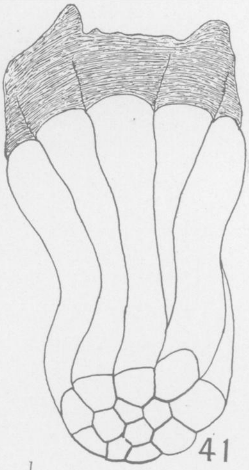

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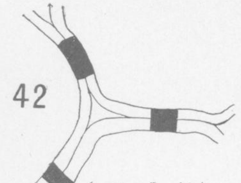

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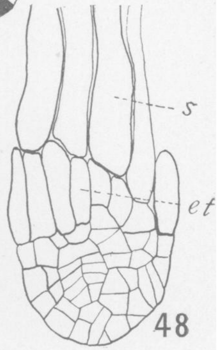

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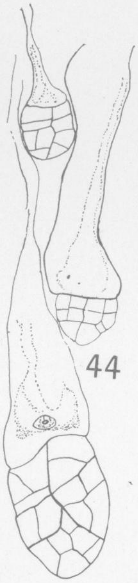

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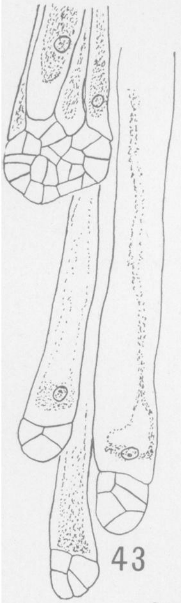

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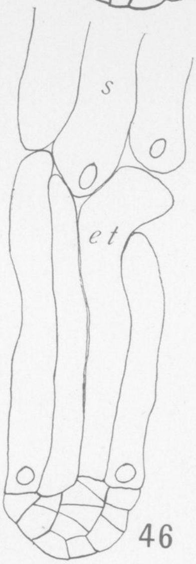

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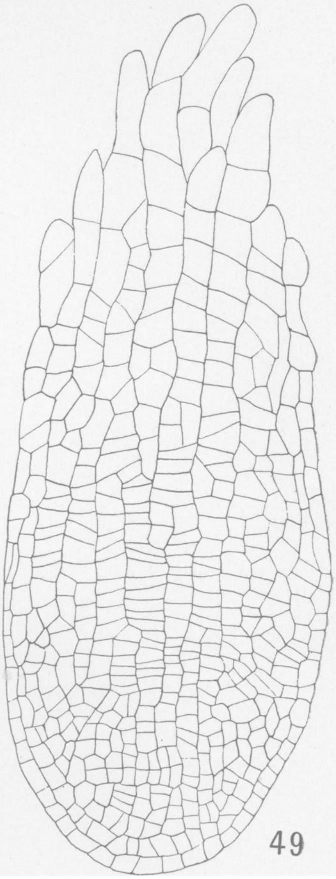

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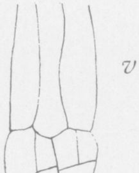

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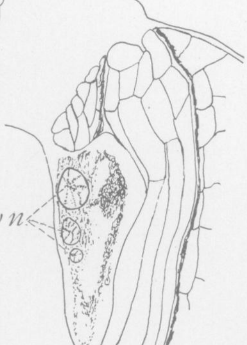

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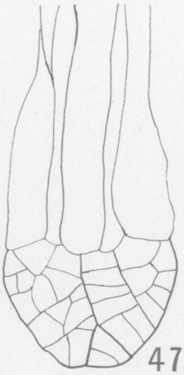

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COKER on PODOCARPUS

pollen grain, were found in material collected from the same tree on a single day. This does not necessarily imply any unusual rapidity of development, for pollen was being shed from this tree for more than a week. As a matter of fact, however, the divisions in the grain, when once begun, follow each other in rapid succession, a fact also noted in the pollen of Pinus by Coulter and Chamberlain (1901). In the same cone undivided grains may be found at the top and three-celled ones at the base. It may be stated at once that there are three mitotic divisions in the pollen grain, forming two prothallial cells, a generative cell, and a tube nucleus. This noteworthy and rather unexpected behavior shows that in all essential points the pollen grains of Podocarpus and of the Abieteae are duplicates, and the peculiarities to be described below do not affect this fundamental agreement.

It is interesting to note here a prediction made by Schacht as long ago as 1860. He says: "Podocarpus, dessen männliche Blüthe den Abietineen entspricht, hat auch dieselbe Form des Blüthenstaubs, der wahrschleinlich in derselben Weise seinen Pollenschlauch entwickelt." The only other gymnosperm in which it is established that two prothallial cells are normally found in the pollen grain is Ginkgo, first figured by Schacht (1860). In *Ceratozamia longifolia* Jurányi (1870) found three small cells (presumably two prothallial and one generative) sometimes present in the pollen grain, although two was the commoner number. In all other Cycadales investigated only one prothallial cell has been found. Schacht found three small cells in the pollen of Ephedra, while Jurányi (1882) figures a varying number, in one case six nuclei being shown. In Taxus and the Cupresseae no prothallial cell has yet been discovered in the microspore, and there are none found in Taxodium.

During all of its divisions the pollen grain of Podocarpus is packed with starch (*figs. 1-6*), which disappears only just before the grain is shed. I know of no other gymnosperm in which this is the case. The first division is shown in *fig. 1*. A prothallial cell of the usual shape is cut off, but, as in other cases, no cellulose wall is formed. The second division follows promptly, cutting off a similar cell (*fig. 2*), and a third gives rise to the generative cell and the tube nucleus (*fig. 3*). The spindles of all the divisions are much broader at the prothallial than at the opposite end, as figured by Coulter and Chamberlain (1901) for Pinus, and the chromosomes are very large in proportion to the spindle (*fig. 3*). Not infrequently this spindle of the third division was strongly inclined (*fig. 6*), and in such cases the generative cell may be placed in a distinctly excentric position.

The prothallial cells do not promptly degenerate as in the Abieteae, but the second, and sometimes the first also, behaves in a manner unknown in other conifers and only paralleled in Ginkgo and the Cycads. Both nuclei organize a coarse reticulum and a nucleolus, and increasing in size become as conspicuous as the generative nucleus (*figs. 3* and *4*).[2] They still remain flattened and elongated, but their size is such that with the generative cell they may occupy half the diameter of the pollen grain (*fig. 5*). So far the development of the two prothallial cells has been the same, but at this point their paths as a rule diverge. The cytoplasm of the second loses its individuality and becomes indistinguishable from that of the tube (or wall) cell, and its nucleus slips from its former position and lies free in the general cytoplasm. This history may be further complicated by the amitotic division of the nucleus before its liberation (*fig. 7*). In such cases the two nuclei generally slip out in different directions and place themselves on opposite sides of the generative cell (*figs. 8* and *10*). Cases were found, however, where both nuclei had moved out in the same direction and lay side by side (*fig. 9*). This was probably the result of an excentric position of the generative cell.

That the division just described is amitotic is proved by many connecting stages, and by the persistent absence of spindles.

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[2] Coulter and Chamberlain (1901) figure both prothallial nuclei as developing a reticulum in *Pinus Laricio*, but this condition is shortly followed by rapid disintegration.

The nucleus before the completion of its division is often much drawn out and thin in the center, as if compressed between the cells above and below. In fact, as the second prothallial nucleus or nuclei move away, the generative cell sinks down and comes in contact with the first prothallial cell (*figs. 7* and *9*). It would be difficult to state the proportional number of cases in which the second prothallial nucleus divides; hundreds of such were found, and in my preparations they were probably as numerous as those in which the division had not occurred.

The first prothallial cell seldom develops further than the stage shown in *fig. 4*. It now begins to degenerate (*fig. 7*), but so slowly that even at the time of shedding it is still conspicuous (*fig. 8*). But while degeneration is the rule for this cell, not a few cases were found where, like the second, it had lost its individuality and liberated its nucleus in the general cavity (*fig. 10*). The nucleus here shows no sign of degeneration, but is apparently as healthy and active as its neighbors. In *fig. 9* two nuclei appear in the first prothallial cell. They are surrounded by dense protoplasm and it is not yet apparent whether they are to be liberated or are to degenerate. In undoubted cases of degeneration the nucleus is often found fragmented into three or more parts.

The generative cell is distinguishable in all cases by its protoplasmic sheath and the structure of its nucleus, which is always denser than any of the other free nuclei of the grain. By comparing the figures it will be seen that the prothallial nuclei after their liberation become much less dense and approach in structure the tube nucleus. They remain more dense, however, than the latter, and are further to be distinguished from it by their size and position.

The question now naturally arises, how much of this history as described is normal and how much is abnormal? The fact that the tree from which this pollen was taken was cultivated under unnatural conditions might lead us to expect abnormalities, and I am inclined to consider as such the fragmentation or liberation of the first prothallial nucleus. But the invariable persistence of the second prothallial nucleus, whether fragmented or not, convinces me that this at least is normal, and that we have in Podocarpus a case analogous in this respect to Ginkgo and the Cycads. This view is further supported by the presence in the pollen tube, in the only three cases in which I was able to follow it throughout, of three small nuclei in addition to the body cell or its products. *Figs. 14* and *16*, sections from the same pollen tube, show a case of this kind. The absence of early stages in the sprouting of the pollen tube makes a distinction between prothallial and stalk nucleus difficult, but their positions favor the designations given in the figures. There can be little doubt as to the identity of the pollen-tube nucleus (*t n*, *fig. 16*).

Jurányi (1870) twice found two nuclei in the tip of the young pollen tube of *Ceratozamia longifolia*, but the doubling may have here resulted from the abnormal conditions, as the tubes were sprouted on ripe pears. Arnoldi (1900, *a*) figures an extra nucleus in the pollen tube of *Cephalotaxus Fortunei*, and very recently Miss Ferguson (1901, *a*) found two cases in which the pollen grain of *Pinus Laricio* still contained three nuclei after the tube nucleus had passed out. In the case of Pinus the most probable explanation seems to be that the extra nucleus is that of the second prothallial cell, as in Podocarpus.

Perhaps the persistence of one of the prothallial nuclei was once common in all conifers; an ancient character which, though now generally suppressed, may occasionally appear. It is of course possible that the particular plant of Podocarpus from which my results were obtained has a peculiar tendency to such a reversion, and that the peculiarities of its pollen are not characteristic for the species. Whether such is the case only future work can determine.

THE POLLEN TUBE.

On June 26, the date of the first collection, fertilization had already taken place in the majority of cases, and only a few undischarged pollen tubes were found. *Fig. 12* illustrates the body cell before its division, what is probably the stalk nucleus lying in contact with it below. Another small nucleus was present in addition to the tube nucleus. In this case the pollen tube had reached the prothallium before the archegonia initials could be distinguished from their neighbors. I have found this to be also the case in both Taxodium and Taxus, when the pollen tubes reach the embryo-sac before the beginning of cell formation. In the Abieteae the tubes reach the archegonia only a few days before fertilization. On reaching the prothallium the pollen tube spreads out to an unusual extent. When only one is present it may cover almost the whole tip of the prothallium and send down short spurs on the sides.

Only two cases were found showing the male cells, but these leave no doubt that there is only one functional male cell formed, as in Taxus. *Figs. 13–16* are sections through the same pollen tube, *13–15* being serial. The male nucleus nearest the tip has appropriated all of the protoplasm, leaving the other thrust almost entirely out of the cell, and naked on its outer surface. The difference in the structure of the two nuclei is also marked. The functional one has the usual structure of the male nucleus in gymnosperms—a very dense, close-meshed linin reticulum, difficult to stain, which, except in very thin sections, almost hides the nucleolus. The latter is not shown in the figures, but appears in another section. In both Podocarpus and Taxodium I can confirm Miss Ferguson's (1901, *a*) statement that there is no metaplasmic substance in the male nuclei. An irregular nucleolus is always present. It is the only part of the nucleus that takes the saffranin stain, and this, together with its evidently compound nature and in Taxodium its behavior in fertilization, convinces me that it is composed of chromatin.

The extruded nucleus shown in *figs. 13* and *14* has apparently gone through the same developmental changes as the functional one, but the reticulum has now begun to coagulate, if the word may be used, into more or less separate clumps, and the evidences of degeneration are unmistakable.

The protoplasm of the male cell consists of a denser inner portion immediately surrounding the nucleus, and an outer sheath which is not at all points sharply distinct from the protoplasm of the pollen tube (*fig. 16*). There is no starch. Goroschankin (1880)[3] found starch in the male cells of the Cupresseae, and Arnoldi (1900) found it in *Sequoia sempervirens*, Cryptomeria, and Taxodium. This I can confirm for Taxodium.

As already stated, the pollen tube was found to contain three nuclei in addition to the male cells.

THE FEMALE PROTHALLIUM.

The macrospore arises deep in the nucellus and is not surrounded by "spongy" tissue such as is found in the Abieteae, Cupresseae, and Taxodieae, and which has so often been erroneously described as of sporogenous character. Miss Ferguson's (1901, *b*) suggestion that the spongy tissue is active in nourishing the prothallium is probably correct; an interpretation I had arrived at from a study of Taxodium.

The youngest stage found is illustrated in *fig. 18*. The prothallium is in the four-celled stage, and at its tip two disorganizing cells are found, which are probably the undeveloped macrospores, but at this late stage it is not safe to draw any conclusion as to their origin. The prothallium is surrounded by cells of the ordinary vegetative type, the innermost of which are disorganizing. The disorganizing cells abut directly on the prothallium, which is not the case where a "spongy" tissue is present.

Stages showing cell formation were not found, but the straight rows of cells conspicuous in young prothallia show that the tissue arises in the usual way by ingrowing tubes. The central region first becomes firm, and in alcohol-fixed material this part remains extended, while the upper and lower buds may be much shrunken. At the time of fertilization the prothallium has reached about two-thirds or three-fourths its full size. *Fig. 21* shows the structure of the prothallium from axis to surface. In the center is a cylinder of very small cells extending from just below the archegonia to near the base. From this row outward the cells increase rapidly in size and become largest near the surface. The outer layer, however, is of an entirely different character. It is composed of very small, regular, epidermis-like cells with dense protoplasm, but almost free from the starch grains found abundantly in other parts. This layer is absent at the tip of the prothallium and is not quite so regular at the base. No such definite layer seems to have been described for other gymnosperms,[4] but in Taxus I have found the outer cells to approach these in character, though not so definitely arranged or so small proportionately. There is little doubt that these surface cells are specially modified for secretion, and it is worth noticing that when a spongy tissue is present there they are not found.

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[3] I have not seen this paper, but take the statement from Arnoldi (1900).

The larger cells of the prothallium, as is usual in conifers, contain many nuclei at the time the embryos are formed (*fig. 20*), while those on the surface have generally only one, or sometimes two.

In only one case were two prothallia found in one ovule (*fig. 19*). Neither had formed archegonia, although the seed had reached its full size. In one of them three or four tracheids were present (*fig. 20*). This will recall the formation of tracheids in the prothallia of ferns in cases of apogamy. In no other case of which I am aware have tracheids been described in the prothallium or endosperms of higher plants.

Hofmeister (1851) found two prothallia in the ovule of *Taxus baccata* and *Pinus silvestris*. He says (p. 127): "Es giebt Bäume von *Pinus silvestris* (ein solcher steht an einer sumpfigen Stelle des Leipziger botanischen Garten), welche, ähnlich der Eybe, in der Mehrzahl ihrer Eychen zwei Embryosäcke entwickeln."[5] Shaw (1896) and Arnoldi (1899) have found it the rule for a number of embryo sacs to be developed in *Sequoia sempervirens*, Arnoldi (1900, *b*) figures five in Cunninghamia, and I have once found two in Taxodium. The case of Gnetum is well known.

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[4] Ikeno (1898) figures smaller and denser surface cells in Cycas, but they do not differ so sharply from the adjoining cells below as in Podocarpus.

[5] Farmer (Annals of Botany **6**:213. 1892) in describing two prothallia in *Pinus silvestris* overlooks this previous discovery by Hofmeister.

THE ARCHEGONIA.

The number of archegonia varies from six or seven to about nine or ten. They are separated from each other by one or more layers of cells, and all open on the upper surface of the prothallium (*fig. 27*). In the youngest stage found, the neck cell was already cut off and had undergone a tangential division (*fig. 22*). In the mature archegonia the neck varies greatly, both in shape and the number of cells (*figs. 23–26*). In one case more than twenty-five cells were counted (*fig. 26*), or there may be only two (*fig. 23*). Perhaps the most common condition is three tiers of four cells each. Murrill (1900) has described considerable variation in the archegonium neck of Tsuga; and in Taxodium also there is much variation.

The jacket cells are not so dense as in many other conifers, and their nuclei do not go to pieces at the maturity of the archegonium, but still retain their shape and structure at the time of fertilization and after (*fig. 31*). Protoplasmic connections between the jacket cells and archegonia were not found, but they were not looked for by special methods, and may be present.

In the position and behavior of the ventral canal nucleus, Podocarpus agrees closely with Taxodium. No membrane is formed separating a ventral canal cell from the egg cell.[6] The nucleus is always closely pressed against the wall of the archegonium at first (*fig. 31*), and in this position it generally remains until the fertilization of the egg. Its position varies greatly. In *fig. 31* it is below the egg nucleus, but it may be placed opposite or above it, but always on the lateral wall and never directly under the neck cells. This retired position has probably been acquired to protect the ventral canal nucleus from harm during fertilization, for, as we shall see, it is to develop further. Both Strasburger (1879) and Belajeff (1893) figure the ventral canal nucleus of Juniperus in a lateral position.[7]

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[6] Arnoldi (1900, *a*) emphasizes the absence of such a membrane in *Cephalotaxus Fortunei*.

[7] Arnoldi (1900, *b*) denies the presence of a ventral canal nucleus in Taxodium, Cryptomeria, Cunninghamia, and Sequoia, and even tries to throw doubt on its occur-

In archegonia which have reached maturity and failed to be fertilized the ventral canal nucleus frequently leaves its position at the surface and moves inward (*fig. 28*). It may even come in contact with the egg nucleus (*fig. 29*). Such cases might be interpreted as evidence in favor of the possible fertilization of the egg by the ventral canal nucleus, as suggested by Coulter and Chamberlain (1901, p. 98). In fact, however, the evidence is in the other direction, for though there is never any obstacle to the free approach of the ventral canal nucleus, and though it is often found very near the egg nucleus, or even in actual contact with it, in no case was an embryo found in an archegonium not provided with a pollen tube. In one case both the egg and ventral canal nuclei had divided amitotically into a large number of fragments, not half of which are shown in the figure (*fig. 30*).

In fertilized archegonia the ventral canal nucleus soon leaves its lateral position, increases in size, and frequently divides amitotically (*figs. 32, 34, 35, 50*). Its reticulum becomes denser and may approach that of the egg nucleus in character (*fig. 35*), as described by Chamberlain (1899) in *Pinus Laricio*. The division of the nucleus, its large size and healthy appearance, and its long persistence (*fig. 50*) leave little doubt that its function is to assist in nourishing the embryo. The persistence of the second male nucleus for a considerable time in the upper part of the archegonium has been described in a number of cases, and I am aware that my figures do not prove conclusively that the nuclei just described do not come from the pollen tube. Degenerating nuclei have been found near the pollen tube (*fig. 33*) which probably come from it, but in all such cases another nucleus was also present. This fact, together with the size, structure, and position of what has been called the ventral canal nucleus, convinces me of the correctness of my interpretation.

rence in the Cupresseae, although both Strasburger and Belajeff have given the spindles of the division in Juniperus. He is certainly mistaken so far as Taxodium is concerned, and I shall be greatly surprised if a ventral canal nucleus is not eventually found in all conifers.

The mature archegonium is shown in *fig. 31*. There are still vacuoles present towards the base, and a mass of kinoplasm completely surrounds the egg nucleus. In the center is another smaller dense area, and at the base a third appears. When the vacuole is nearer the center of the archegonium, only two kinoplasmic masses are present, one above and one below. The upper one does not always completely surround the nucleus, but is often only in contact with it at the side. As I have described these masses in some detail in Taxodium, and as they seem almost identical in the two genera, they need not detain us here.

At the tip of the egg cell just under the neck a collection of plastic material is figured. It stains red in saffranin and may easily be mistaken for a disorganizing ventral canal cell. It is generally present in both Podocarpus and Taxodium.

The pollen tube enters the neck, penetrates for some distance into the archegonium, and discharges its contents into the egg (*fig. 17*). The opening in the pollen tube is distinctly seen in *fig. 32*, where some of the protoplasm of the egg seems to have surged up through it after the discharge. The archegonium wall does not show the thickening around and below the neck characteristic of the Cupresseae (among which Taxodium must be placed), but is thin throughout and permits of much extension by the pollen tube (*figs. 32, 33*).

Strasburger (1892) has described the reduction of one male cell in Taxus and the Abieteae when the pollen tube fertilizes only one archegonium, and the recent work of Murrill (1900) and Miss Ferguson (1901, *a*) confirms this.[8] Where the archegonia are collected into complexes, the pollen tubes do not enter their necks, and both male cells are functional.

THE EMBRYO.

Fertilization stages were not found, but from *fig. 32* it seems probable that the fusion nucleus moves to the base of the archegonium before dividing, as in Juniperus (Strasburger, 1879), Taxus (Jäger, 1899), Taxodium,[9] Cryptomeria, and Sequoia

[8] Cephalotaxus (Arnoldi, 1900, *a*) is a possible exception.

[9] Noted for Taxodium in my preliminary paper, *l. c.*

(Arnoldi, 1900, *b*). The two nuclei here lie side by side, and are not surrounded by starch or furnished with so distinct a protoplasmic sheath as in the Cupresseae and Taxodieae. A denser and more granular area can be observed in immediate contact with the nuclei, but it is not sharply defined and fades imperceptibly in the general protoplasm of the archegonium. In *fig. 33* the second division has just occurred, and the nuclei, only two of which are shown, have not yet reached their full size. When the sixteen-celled stage is reached, the protoplasm of the embryo has become separated from the disorganizing area above (*fig. 34*). There is probably no further division before cell walls are formed, but the absence of important stages here leaves this point in doubt.

Fig. 38 shows the proembryo just as it is breaking through the base of the archegonium. It consists here, as invariably, of three tiers. First is a rosette of generally fourteen nuclei, which are separated from one another by cell walls, but are in open communication with the archegonium above; next come the suspensors, also usually fourteen in number, and below there is the large tip cell, with two nuclei not yet separated by a wall.

It will be noticed that the walls between the rosette nuclei seem to continue those separating the suspensors below. This is strong evidence that the rosette and the suspensors are established only after cell walls are formed, through the division of an originally single upper tier. It is in this way that they are formed in Taxodium. Between rosette and suspensors a thick cellulose plug is soon formed, a novelty, I believe, in the gymnosperm embryo (*figs. 38, 39, 41*). As the suspensors elongate, their nuclei, contrary to the rule, remain for some time at the archegonial end (*fig. 39*). This is probably connected with the deposition of the cellulose plug. The first division of the tip cell is longitudinal (*fig. 40*); and this is probably followed by another longitudinal wall in each cell, giving four tip cells in a tier. Such a stage was not found, but the frequent splitting of the proembryo into four parts suggests it. In *fig. 44* three embryos of such a group are shown, each at the tip of a single suspensor. In *fig. 43* the three lower embryos have come, with one other, from a single archegonium, while the upper larger one is from another archegonium, in this case the suspensors not having separated. Fourteen suspensors appear in cross section in *fig. 36* and thirteen in *fig. 41*. As the proembryo penetrates further and further into the prothallium a number of the suspensors drop out at different levels, and cross sections just above the tip cells show a greatly reduced number (*fig. 37*). Jäger (1899) has described a similar behavior in Taxus. If the tip cells separate, each is usually furnished with a single suspensor (*figs. 43, 44*). Protoplasmic connections occur between the suspensor cells near the archegonium (*fig. 42*). Although the individual fibers were scarcely distinguishable, the appearance leaves no doubt of their presence.

The embryonal tubes may appear very early (*fig. 46*), but usually not before the stage shown in *fig. 48*. They are formed by the almost simultaneous elongation of all the cells on the proximal surface of the embryo, and by their growth force it deeper into the prothallium.

Various stages in the development of the embryo are given in *figs. 43–48*. If the suspensors do not separate, each of the four-tip cells generally divides first by a transverse wall; if they do separate, the first division of the single tip cell is generally longitudinal. Further than this no regularity is apparent.

A median longitudinal section of the oldest embryo found is given in *fig. 49*. There is no indication as yet as to where the root tip is to appear, and a distinction between dermatogen, periblem, and plerome has not arisen.

In one case an embryo was found which had grown directly upward and passed out between the prothallium and nucellus (*fig. 50*), where, bending sharply, it continued its course for some distance.

AFFINITIES OF PODOCARPUS.

In looking over the results of this paper, we find that Podocarpus agrees with the Abieteae in the gross structure of the pollen grain (long known, of course), in the presence of two male prothallial cells, in the distribution and arrangement of the archegonia, in the reduction of the number of functional male nuclei to one (?), and in the penetration of the pollen-tube into the archegonium. The first three of these characters, at least, are of much taxonomic importance, as is shown by their constancy in the different groups. On the other hand, the structure and manner of formation of the proembryo, where points of difference appear, although constant, so far as known in the Abieteae,[10] are by no means so constant in other families, and are not of such value in classification.

When it is noted further that none of the characters of agreement mentioned are found in the Cupresseae, and few of them in any other conifers, it seems safe to conclude that in the Podocarpeae are to be found the nearest living relatives of the Abieteae.

SUMMARY.

1. The pollen grain contains two prothallial cells.
2. The second prothallial nucleus persists and is found later in the tip of the pollen tube. It may divide amitotically.
3. The mature pollen grain sometimes contains as many as six nuclei, probably abnormally.
4. The pollen tube reaches the prothallium before the archegonial initials can be distinguished. It contains no starch.
5. There is but one functional male cell formed.
6. There is no "spongy" tissue around the macrospore.
7. The prothallium is furnished with a distinct epidermis-like layer of small regular cells which are modified for secretion.
8. Two large prothallia were once found in the same ovule. One of them contained tracheids.
9. The archegonia vary from about seven to eleven, and all open on the micropylar end of the prothallium. The neck varies greatly in shape and number of cells. Two large kinoplasmic

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[10] Only the interarchegonial development is referred to here. Many variations are recorded in its later history.

masses are generally present in the mature archegonium, one in contact with or surrounding the egg nucleus, the other near the base.

10. The ventral canal nucleus is not separated from the protoplasm of the egg by a membrane. It persists for some time after fertilization and probably assists in nourishing the embryo.

11. The pollen tube enters the archegonium to discharge its contents.

12. Four divisions of the fusion nucleus occur before cell walls are formed.

13. The proembryo consists of three tiers of cells, the upper two of fourteen each, the lower of one cell containing two nuclei.

14. A heavy cellulose plug is formed between the suspensors and the rosette.

15. The suspensors may separate and several embryos develop from one archegonium.

16. The Podocarpeae are considered as the nearest living relatives of the Abieteae.

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EXPLANATION OF PLATES V-VII.

The figures, with the exception of *fig. 19*, were drawn with Leitz camera lucida, and reduced one-half by photography. The magnification given in each case is one-half that of the original drawing. Figures from *Podocarpus coriacea* are so designated. All others are from material collected in Darlington, S. C., from a species probably the same, but not certainly identified. The abbreviations used are: *b c*, body cell; *dn*, degenerating male nucleus; *e n*, egg nucleus; *e t*, embryonal tubes; *g*, generative cell; *m c*, male cell; *p^1*, 1st prothallial cell, or the two nuclei arising from the division of its nucleus; *p^2*, 2d prothallial cell, or the two nuclei arising from the division of its nucleus; *r*, rosette cells; *s*, suspensors; *stn*, stalk nucleus; *tn*, tube nucleus; *v n*, ventral canal nucleus, or group of nuclei arising from its division.

PLATE V.

FIG. 1. First division of pollen grain. × 1550.

FIG. 2. Second division of pollen grain. × 1550.

FIG. 3. Third division of pollen grain. × 1550.

FIG. 4. The same, later stage. × 1550.

FIG. 5. Pollen grain soon after third division; starch still present. ×750.

FIG. 6. Oblique spindle of third division. × 750.

FIG. 7. Pollen grain soon after the amitotic division of the second prothallial nucleus. × 1550.

FIG. 8. Five nuclei in pollen grain, two of which have arisen from the amitotic division of the second prothallial nucleus. × 750.

FIG. 9. Pollen grain with six nuclei; the first prothallial nucleus has also divided amitotically. × 750.

FIG. 10. Pollen grain with five nuclei, all of which are lying free in the general cytoplasm: the generative nucleus is furnished here, as always, with a special protoplasmic sheath. × 750.

FIG. 11. Section of wall of microsporangium just before the first division of the pollen grain. × 315.

FIG. 12. Body cell and stalk nucleus, after the pollen tube has reached the prothallium. × 315.

FIGS. 13–16. Sections through the same pollen tube, showing functional male cell, degenerating male nucleus, and three small nuclei; *figs. 13, 14,* and *15* are consecutive. × 315.

FIG. 17. Diagram showing pollen tube from which the above figures mere made; other pollen tubes and a fertilized archegonium are also shown. × 30.

FIG. 18. Four-celled female prothallium with two disorganizing microspores at tip. × 150. *P. coriacea.*

FIG. 19. Diagram of two mature prothallia in the same nucleus; the cross indicates the position of the tracheids. × 4½. *P. coriacea.*

FIG. 20. Tracheids found in one of the prothallia represented in the above diagram; they are surrounded by multinucleate cells of the usual type. × 150. *P. coriacea.*

PLATE VI.

FIG. 21. Median longitudinal section of prothallium from center to surface, showing small cells of the axial rows, and a single layer of small epidermis-like surface cells. × 30.

FIG. 22. Young archegonium; neck cell shows a cross division. × 150. *P. coriacea.*

FIGS. 23–26. Neck cells of mature archegonia, showing great variation. × 150.

FIG. 27. Diagram of tip of ovule; archegonia mature. × 30.

FIG. 28. Egg nucleus and ventral canal nucleus in mature archegonium. × 150.

FIG. 29. Egg nucleus and ventral canal nucleus in contact. × 315.

FIG. 30. Numerous nuclei which have arisen from amitotic division of the egg and ventral canal nuclei in the tip of the archegonium. × 315.

FIG. 31. Mature archegonium; ventral canal nucleus pressed against the wall below the egg nucleus; three kinoplasmic masses present, one surrounding the egg nucleus; at the tip is a collection of plastic material. × 150.

FIG. 32. Two-celled embryo in base of archegonium; ventral canal nucleus above. × 150.

FIG. 33. Four-celled embryo; pollen tube still visible above, with a nucleus near its tip. × 150.

FIG. 34. Sixteen-celled embryo; several nuclei in the disorganizing cytoplasm above. × 150.

FIG. 35. Two nuclei in tip of an archegonium in the base of which is an embryo in the stage shown in *fig. 38*. × 315.

FIG. 36. Cross section of a group of fourteen suspensors. × 315.

FIG. 37. Cross section of a group of five suspensors just above the tip cells. × 315.

PLATE VII.

FIG. 38. Young embryo breaking out of archegonium. × 315.

FIG. 39. Slightly older embryo; two nuclei in one tip cell. × 315.

FIG. 40. Still older embryo, with two tip cells, one of which contains two nuclei. × 315.

FIG. 41. A group of thirteen suspensors; at their archegonial end is the thick cellulose plug. × 315.

FIG. 42. Protoplasmic connections between adjoining suspensors of a group. × 1550.

FIG. 43. A group of embryos from two archegonia. × 150.

FIG. 44. Another group of embryos from a single archegonium. × 150.

FIG. 45. Young embryo before development of embryonal tubes. × 150.

FIG. 46. Embryonal tubes, developed unusually early. × 150.

FIG. 47. Embryo just before formation of embryonal tubes. × 150.

FIG. 48. Origin of embryonal tubes. × 150.

FIG. 49. Older embryo. × 150.

FIG. 50. Abnormal embryo, growing upwards and passing between prothallium and nucellus. × 150.